IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V., a corporation organized under the laws of Germany; et al., Plaintiffs, v. WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH, a Delaware corporation; et al., Defendants.	Civil Action No. 1:09-cv-11116-PBS

DECLARATION OF HELEN LOCKHART

I, Helen Lockhart, depose and state as follows:

1. I am an attorney and a shareholder with the firm Wolf, Greenfield & Sacks, P.C.

2. I have represented the Whitehead Institute for Biomedical Research ("Whitehead Institute") since approximately 2002. In early 2004, I began representing the Whitehead Institute in connection with the prosecution of the patent applications that Plaintiffs refer to as the Tuschl I applications, which are co-owned by Whitehead Institute, Massachusetts Institute of Technology ("MIT"), University of Massachusetts ("UMass"), and Max-Planck-Gessellschaft Zur Förderung Der Wissenschaften E.V. ("Max Planck").

3. I have been continuously involved in the prosecution of the Tuschl I applications since that time. In the course of prosecuting the Tuschl I applications, it has been my practice to provide all of the co-owners, including Max Planck, drafts of submissions we have intended to

file with any patent office before we have filed the final documents, as well as copies of the final version of the submissions.

4. I have reviewed Plaintiffs' Motion for Preliminary Injunction, Memorandum in Support of Max Planck's and Alnylam's Motions for Temporary Restraining Order and Preliminary Injunction, and the Affidavits of Dr. Joern Erselius, Dr. Wolfgang Weiss, Dr. Nancy J. Linck, David I. Gindler, and Dr. Sandra L. Haberny and the Amended Affidavit of Joern Erselius filed in the above-captioned matter.

July 11, 2005 Information Disclosure Statement

5. I am familiar with the July 11, 2005 Information Disclosure Statement ("IDS"), attached hereto as Exhibit 1 (this is the same document attached as Ex. F to the Amended Affidavit of Joern Erselius), and referred to in the Affidavits of Joern Erselius ("Erselius Affidavit"), Nancy J. Linck, and Sandra L. Haberny and the Amended Affidavit of Joern Erselius ("Erselius Amended Affidavit"). This IDS was submitted to the Patent Office in connection with the prosecution of U.S. Patent App. No. 09/821832 ("the '832 application"), one of the Tuschl I applications. I was personally involved in the drafting and filing of this IDS.

6. This IDS was filed at the request of Max Planck's counsel at the Rothwell Figg firm, who provided a first draft of the IDS, which was then revised by the Whitehead Institute prior to filing.

7. I have reviewed the June 30, 2009 Affidavit submitted by Dr. Linck ("Linck Affidavit"). In that Affidavit at paragraph 18, Dr. Linck states that in the July 11, 2005 IDS, "the Tuschl I applicants state that they did not invent 'the aforementioned therapeutically advantageous features' that are actually part of the Tuschl II invention." This is not an accurate description of the July 11, 2005 IDS.

8. First, although the Linck Affidavit puts quotation marks around the phrase "the aforementioned therapeutically advantageous features," the July 11, 2005 IDS does not include that language.

9. In addition, contrary to the Linck Affidavit, the IDS does not state that the Tuschl I applicants did not "invent" any invention.

10. The July 11, 2005 IDS stated only that "[t]he subject matter of the text at page 14 line 25 – page 15 line 13 was previously included" in an earlier Tuschl II European patent application. *See* Exhibit 1 (July 15, 2005 IDS) at 2. The IDS also stated that "Applicants do not rely on the above-described text for supporting any of the currently pending claims." *Id.* The IDS also stated that the Applicants were not relying on Example 5 "for supporting any of the currently pending claims" of the '832 application, and that "the data of Example 5 was included" in the Tuschl II application serial no. 10/433,050, which claims priority to the Tuschl II European Patent Application No. 00126325.0 ("Tuschl II '325 application"). *Id.*

11. The purposes of filing the July 11, 2005 IDS were: (1) to make sure that the PTO was fully aware of all information that a reasonable examiner might find material to the patentability of the claims of the '832 application; and (2) to address an issue raised by Max Planck regarding whether the PTO had all relevant information. The July 11, 2005 IDS communicated to the PTO that the data described in the identified portions of the '832 application were generated by a group of persons that might have included persons beyond the inventors of the '832 application, and that the '832 applicants were not relying on the text describing that data to support the claims of the '832 application. This language was not in any way intended to communicate, nor did it communicate, that the identified portions of the '832 application do not belong in the application.

12. The July 11, 2005 IDS did not state that the data described in the specific portions of the '832 application constitutes a patentable invention and, thus, did not state that the '832 applicants did not "invent" that data. *See* Exhibit 1 (July 15, 2005 IDS).

13. I have reviewed the June 30, 2009 Affidavit submitted by Dr. Sandra L. Haberny ("Haberny Affidavit"). The Haberny Affidavit states at paragraph 6 that the July 11, 2005 IDS refers to the same portions of the '832 application referred to above, plus Figure 14, and states that in the July 11, 2005 IDS the '832 applicants "admi[tted] . . . that they did not invent nor do they own that data." This is not an accurate description of the July 11, 2005 IDS.

14. As stated above, the July 11, 2005 IDS did not state that the data described in the relevant portions of the '832 application constituted an invention, and it made no statement concerning who "invented" that data. *See* Exhibit 1 (July 15, 2005 IDS). Moreover, the July 11, 2005 IDS did not state that the '832 applicants did not own the data referred to. *Id.*

15. I have reviewed the June 30, 2009 Erselius Affidavit as well as the July 8, 2009 Erselius Amended Affidavit. The Erselius Amended Affidavit asserts at paragraph 20 that the July 11, 2005 IDS "affirmatively represent[ed] to the USPTO that the assignees of the Tuschl I applications do not own the Tuschl II inventions, and that the Tuschl I applicants do not rely on the Tuschl II inventions to support the inventions being pursued in the Tuschl I applications." This is not an accurate description of the July 11, 2005 IDS.

16. As stated above, the July 11, 2005 IDS did not state that the data at issue constituted an invention, and thus did not state that the data constituted a "Tuschl II invention." *See* Exhibit 1 (July 15, 2005 IDS). The July 11, 2005 IDS also made no statement that the assignees of the '832 application "do not own" the data in the '832 application. *Id.*

June 18, 2009 Submission

17. I was personally involved in drafting and filing a submission made by the Tuschl I applicants in the prosecution of a Tuschl I application (U.S. Patent Application No. 10/255568) ("the '568 Tuschl I application") that was submitted on June 18, 2009. This June 18, 2009 submission is attached as Exhibit 2 (the same document is Exhibit I to the Erselius Amended Affidavit). The Erselius Amended Affidavit at paragraph 24 also refers to this submission.

18. The Erselius Amended Affidavit states that this submission "implicitly argued that the USPTO should reject the Tuschl II applications for containing the same inventions as the Tuschl I applications" and that "Whitehead neither consulted nor informed me or anyone at Max Planck Society or Max Planck Innovation before submitting this argument to the USPTO." Erselius Amended Affidavit, ¶ 24. This is not an accurate statement.

19. In accordance with my general practice discussed above, on January 21, 2009, I provided a draft of what became the June 18, 2009 submission to Max Planck. A copy of this draft is attached as Exhibit 3. The draft included responses to the rejection of certain claims of the '568 Tuschl I application on double-patenting grounds over certain claims of both certain Tuschl II applications and of another Tuschl I application: the '832 Tuschl I application. The draft also included the arguments regarding priority and 35 U.S.C. § 112.

20. The argument addressing the rejection of the '568 claims over certain '832 Tuschl I claims consisted of a statement that a terminal disclaimer had been filed to overcome this double-patenting. We asked Max Planck to agree to file such a disclaimer. Max Planck did not agree to do so. Nor did it provide any comments on the draft submission that I provided to them.

21. When we did not receive any comments from Max Planck, we finalized the draft submission and filed it with the PTO. Because Max Planck refused to agree to file a terminal

disclaimer to overcome the double-patenting rejection over claims of the '832 Tuschl I application, the filed version of the submission included a final argument which was not circulated to Max Planck before filing. This final argument, however, did not concern the rejection of the '568 claims over the Tuschl II applications; rather, it concerned the rejection of certain '568 clams over the '832 Tuschl I application. Thus, it did not affect the rights of the Tuschl II applicants. The submission also included a paragraph on page 9 addressing the priority date of the '568 application that was similar to a paragraph on page 12 in a prior submission during prosecution to which Max Planck / Alnylam had agreed. This prior January 5, 2007 submission is attached as Exhibit 4, and a copy of a December 7, 2006 email from Alnylam to Wolf Greenfield agreeing to the language in this submission is attached as Exhibit 5.

22. Prior to making the submission to the PTO on June 18, 2009, Whitehead was aware of Max Planck's position concerning the Tuschl I applications – namely, that the Tuschl I applicants should delete the text identified in the Haberny Affidavit from the Tuschl I applications and delete the claim of priority to the Tuschl II '325 application.

23. These issues concerning the prosecution Tuschl I applications have been the subject of debate between Max Planck and Whitehead, as well as UMass and MIT, for at least the past five years.

Speculative Statement in Linck Affidavit Regarding Tuschl I Allowance

24. The Linck Affidavit, at paragraph 25, states that "it appears likely" that a Tuschl I application will issue. I understand that Plaintiffs in this case assert that "[i]t now appears that [the PTO] will very shortly give notice that it intends to issue a patent based on a Tuschl I application . . . ." Pls' Mem. at 2. I believe these statements are speculative.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: July 14, 2009 /s/ Helen Lockhart

Helen Lockhart, Ph.D.[1]

Certificate of Service

I, Christopher M. Morrison, hereby certify that I have this 14th day of July 2009 served a true copy of the foregoing document to the registered participants as identified on the Notice of Electronic Filing.

/s/ Christopher M. Morrison
Christopher M. Morrison

[1] Pursuant to District of Massachusetts Electronic Case Filing Administrative Procedure J(3), the filing attorney shall retain the original for future production, if necessary, for two years after the expiration of the time for filing a timely appeal.

EXHIBIT 1

DOCKET NO.: W0571.70010US02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Thomas Tuschl et al.
Serial No.:	09/821,832
Confirmation No.:	6240
Filed:	March 30, 2001
For:	RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE
Examiner:	S. Chunduru
Art Unit:	1637

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to MAIL STOP RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 7th day of July, 2005.

Helen C. Lockhart, Ph.D., Reg. No. 39,248

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 CFR §§1.56, 1.97 AND 1.98

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§1.56, 1.97 and 1.98, the Applicant requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. §1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action after the filing of a request for continued examination under 37 C.F.R. §1.114.

No fee or certification is required.

PART II: Information Cited

The Applicant hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified). The order of presentation of the references should not be construed as an indication of the importance of the references.

PART III: Other Information:

In the interest of insuring that the Examiner is aware of all relevant facts known to the Applicants, the Examiner's attention is directed to the existence of a co-pending patent application, US Application Serial No. 10/433,050.

The present application contains text at page 14 line 25 – page 15 line 13 and in example 5, which was not included in US provisional patent application 60/193,594 to which the above-identified patent application claims priority.

The subject matter of the text at page 14 line 25 – page 15 line 13 was previously included in European Patent Application No. 00126325.0 filed in the names of Tuschl, Elbashir, and Lendeckel, to which US Application Serial No. 10/433,050 claims priority (copies enclosed). Thomas Tuschl, one of the named co-inventors of European Patent Application No. 0 0126325.0 and US Application Serial No. 10/433,050 is a co-inventor of the present patent application. Elbashir, and Lendeckel are not named inventors on the present patent application. Applicants do not rely on the above-described text for supporting any of the currently pending claims. Additionally, the present application contains Example 5. Applicants do not rely on the above-described Example for supporting any of the currently pending claims. Rather, Applicants contend that the present claims are fully allowable without reliance upon such information. The data of Example 5 was included in US10/433,050 which claims priority to European Patent Application No. 00126325.0.

Also enclosed is Exhibit 1 of IDS. This document was filed against the corresponding Japanese application.

PART IV: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed form PTO-1449 be signed by the Examiner to evidence that the cited information has been fully considered by the Patent and Trademark Office during the examination of this application;

3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the Applicant makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

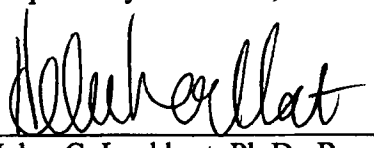
By submitting this Information Disclosure Statement, the Applicant makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. §1.56(b).

By submitting this Information Disclosure Statement, the Applicant makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. §102.

Notwithstanding any statements by the Applicant, the Examiner is urged to form his own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

Respectfully submitted,

By: 

Helen C. Lockhart, Ph.D., Reg. No. 39,248
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Docket No.: W0571.70010US02
Date: July 7, 2005
x07/07/2005x

EXHIBIT 2

Docket No.: W0571.70010US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Thomas Tuschl et al.
Serial No.:	10/255,568
Confirmation No.:	2920
Filed:	September 26, 2002
For:	RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE
Examiner:	L. V. Wollenberger
Art Unit:	1635

Certificate of Electronic Filing Under 37 CFR 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).
Dated: 6-16-09 Signature: Janet D'Annunzio-Ellis (Janet D'Annunzio-Ellis)

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Office Action mailed December 18, 2008, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikethroughs or double bracketing.

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1-16. (Canceled)

17. (Previously Presented) A method of mediating RNA interference of an mRNA in a cell in vitro comprising:

(a) introducing into the cell isolated double stranded RNA of from about 21 nucleotides to 23 nucleotides in length, wherein the double stranded RNA is in the form of two separate strands which are not covalently linked and has sequence correspondence to the mRNA and wherein the double stranded RNA mediates RNA interference by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within the region of sequence correspondence with the double stranded RNA, and wherein the mRNA is mammalian cellular mRNA;

(b) maintaining the cell produced in (a) under conditions such that degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA in the cell.

18. (Canceled)

19. (Canceled)

20. (Currently Amended) A method of mediating RNA interference of an mRNA in a cell in vitro, comprising:

(a) combining isolated double-stranded RNA, wherein the strands are not covalently linked, that corresponds to a sequence of the mRNA with a soluble extract that mediates RNA interference, thereby producing a combination, wherein the soluble extract is derived from syncytial blastoderm Drosophila embryos;

(b) maintaining the combination produced in (a) under conditions such that the double-stranded RNA is processed to double stranded RNA of from about 21 to about 23 nucleotides, thereby producing double stranded RNA of from about 21 to about 23 nucleotides;

(c) isolating double stranded RNA of from about 21 to about 23 nucleotides produced in (b);

(d) introducing double stranded RNA isolated in (c) into the cell; and

(e) maintaining the cell produced in (d) under conditions such that degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA in the cell.

21. (Canceled).

22. (Previously Presented) The method of Claim 20, wherein the double stranded RNA of (c) is isolated using gel electrophoresis.

23. (Previously Presented) A method of mediating RNA interference of an mRNA in a cell in vitro, comprising: (a) introducing into the cell isolated double stranded RNA of from about 21 nucleotides to 23 nucleotides in length, wherein the double stranded RNA is in the form of two separate strands which are not covalently linked and has sequence correspondence to the mRNA, wherein the double stranded RNA is chemically synthesized and wherein one or more nucleotides of the double stranded RNA are a non-naturally occurring nucleotide or deoxyribonucleotide or non-standard nucleotide, and wherein the double stranded RNA mediates RNA interference

by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within the region of sequence correspondence with the double stranded RNA, and wherein the mRNA is mammalian cellular mRNA and (b) maintaining the cell that contains the double stranded RNA such that degradation of the mRNA occurs, thereby mediating RNA interference of mRNA in the cell.

24.-75. (Canceled)

76. (Currently Amended) The method ~~of any one of claims 17, 20 and~~ of claim 23, wherein the mRNA is human mRNA.

77.-79. (Cancelled)

80. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is from 21 to 23 nucleotides in length.

81. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 21 nucleotides in length.

82. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 22 nucleotides in length.

83. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 23 nucleotides in length.

84. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is perfectly complementary to the mRNA.

85. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA comprises a terminal 3' hydroxyl group.

86. (New) The method of claim 17, wherein the double stranded RNA is from 21 to 23 nucleotides in length.

87. (New) The method of claim 17, wherein the double stranded RNA is 21 nucleotides in length.

88. (New) The method of claim 17, wherein the double stranded RNA is 22 nucleotides in length.

89. (New) The method of claim 17, wherein the double stranded RNA is 23 nucleotides in length.

90. (New) The method of claim 17, wherein the double stranded RNA is perfectly complementary to the mRNA.

91. (New) The method of claim 17, wherein the double stranded RNA comprises a terminal 3' hydroxyl group.

92. (New) The method of claim 17, wherein the mRNA is human mRNA.

93. (New) The method of claim 20, wherein the mRNA is human mRNA.

REMARKS

Applicant respectfully requests reconsideration. Claims 17, 20-23, 76 and 80-85 were previously pending in this application. Claims 76 and 80-85 have been amended to limit the dependency to claim 23. New claims 86-93 have been added to add the subject matter of claims 76 and 80-85 as they depend from claims 17 or 20. Claim 20 is amended herein to add the limitation of former claim 21. Claim 21 is canceled. As a result, claims 17, 20, 22-23, 76, and 80-93 are pending for examination with claims 17, 20, and 23 being independent claims. No new matter has been added.

Interview Summary

Applicants thank Examiner Wollenberger for the courtesy of conducting a telephone interview on December 16, 2008. During the interview double patenting rejections of pending claims and written description rejection of claim 20 were discussed.

Withdrawn Rejections

The Examiner is thanked for the indication that the rejection of the claims under 35 USC 102(e) as being anticipated by Fire et al. is withdrawn. Applicant confirms the Office position that "about 21 nucleotides to 23 nucleotides" in independent claims 17 and 23 is considered to embrace dsRNA in which each strand has a length ranging in size from slightly shorter than 21 nucleotides to no longer than 23 nucleotides.

Priority

The Examiner has accorded an earliest effective filing date of January 31, 2001 to claims 23, 76, and 80-85. The other claims were accorded an earliest effective filing date of the provisional application filed March 30, 2000. Applicant disagrees with the assignment of the later priority date to claims 23, 76, and 80-85. US 60/193594 filed on March 30, 2000 describes the use of "altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides (e.g., analogs)." It was clear to the skilled artisan, at the time

the priority document was filed, that the cited language referred to the use of non-standard nucleotides. The ipsis verbis recitation of the term non-standard nucleotide is not required to provide an adequate written description of the invention. (See for instance MPEP 2163 II(a)3(a).)[1] At the time the application was filed it was understood in the art that standard nucleotides include A, C, T, G, and U. It was also understood that numerous other non-standard nucleotides exist and were useful in certain DNA and RNA based systems. For instance, US Patent 5,965,364 to Benner entitled "Method for selecting functional deoxyribonucleotide derivatives" which issued in 1999, before the priority date of the instant patent application, discloses the use of non-standard nucleotides. US Patent 5,965,364 describes the use of non-standard nucleotides or nucleobases in nucleic acid based libraries. It is pointed out in the patent that conventional nucleotides are "ribo or deoxyribo adenylic acid, guanylic acid, and cytidylic acid, and uridylic acid or thymidylic acid, referred to by convention as A, G, C, U and T."

The term non-standard nucleotide is also described in US Patent No. 6,127,535, issued in 2000 and having a filing date in 1998. It is taught in US 6,127,535 that "The term 'nucleotide' as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. Nucleotides generally include a base, a sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; all hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as recently summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183."

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[1] "If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient")."

Non-standard nucleotides are also described in the non-patent literature prior to Applicant's priority date. For instance, Lutz, et al, Differential discrimination of DNA polymerase for variants of the non-standard nucleobase pair between xanthosine and 2,4-diaminopyrimidine, two components of an expanded genetic alphabet, Nucleic Acids Res. 1996 April 1; 24(7): 1308–1313, copy attached hereto, describes the use of non-standard nucleotides.

A person of ordinary skill would have understood, at the time the patent application was filed, that altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides (e.g., analogs) refers to non-standard nucleotides, i.e. nucleotides other than standard A, C, T, G, and U nucleotides. In view of the knowledge of the skilled artisan at the time of the invention one skilled in the art also would have been able to make and use dsRNA, as described in the instant specification, with non-standard nucleotides. Thus, US provisional patent application 60/193594 filed on March 30, 2000 provides an enabling and adequate written description for the term "non-standard nucleotide".

In order to clarify the record, Applicant has rewritten dependent claims 76 and 80-85 as they depend from claims 17 and 20 as separate dependent claims. The record is clear that new claims 86-93 are entitled to the earliest priority date associated with US 60/193594, March 30, 2000.

Rejection under 35 USC 112

Claim 20 has been rejected under 35 USC 112 first paragraph as failing to comply with the written description requirement. Although Applicant disagrees with the rejection, in order to advance prosecution, Applicant has amended claim 20 to incorporate the limitations of pending claim 21. Claim 21 has not been rejected. It is believed that the amendment is sufficient to overcome the rejection.

Double Patenting Rejection

Claims 17, 20, 23, 76 and 80-85 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 50-127 of copending Application No. 10/832,257. Claims 17, 20-23, 76 and 80-85 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable

over claims 48, 49, 51, 53-57, 60-64, 67-73 and 75-125 of copending Application No. 10/433,050. Claims 17, 23, 76 and 80-85 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 30 and 34-55 of copending Application No. 11/142,866. Applicants respectfully request reconsideration.

The rejection has been maintained over each of US 10/832257, 10/433050 and 11/142866 because the double patenting rejection is not the last remaining rejection. It is believed that in view of the above-arguments and amendments, the double patenting rejection will be the last remaining rejection in the case. The instant application has an earlier effective priority date than each of US 10/832257, 10/433050 and 11/142866. Thus, it is requested that the rejection be withdrawn and the instant claims be allowed. For the sake of completeness Applicants present arguments below to address the substance of the double patenting rejection.

The Examiner states that since both applications have one inventor in common a non-statutory double-patenting rejection is properly maintained. The Examiner has disagreed with Applicants' arguments that common ownership means wholly owned by the same person or organization. Applicants respectfully request reconsideration.

Applicants previously argued that the double patenting rejection was not appropriate because the two applications are not "commonly owned". Common ownership is defined in MPEP 706.02(l). MPEP 706.02(l) states that the "term 'common ownership' means wholly owned by the same person(s) or organization(s) at the time the invention was made." The instant patent application is owned by four entities and US Patent Applications No. 10/832,257; 11/142,866 and 10/433,050 are owned by only one of the four entities. The Examiner has dismissed these arguments because the definition of "common ownership" is found in a section of the MPEP (706.02(I)) which "is directed to issues having to do with establishing common ownership under 35 USC 103(c) and does not directly address the particular problems of dual ownership of two or more patents to the same or obvious variations of the same invention, as is the case here." (Office Action page 7). However, MPEP 706.02(I) describes common ownership with respect to obviousness under 35 USC 103(c) as well as double patenting (See MPEP 706.02(I)(2) & (3)). Additionally, the case law supports Applicants' interpretation that double patenting requires common ownership. For instance the court in *In re Longi* (759 F.2d 887, 896, 225 USPQ 645, 651 (Fed. Cir. 1985))

addressed the issue that a double patenting rejection is appropriate over a patent having a different inventive entity if common ownership existed. Additionally Applicant is not aware of a definition of common ownership in the case law or the MPEP that contradicts the definition of common ownership found in MPEP 706.02(I).

As the Examiner is undoubtedly aware, the appropriate legal analysis for obviousness type double patenting requires a determination that the differences between the claimed inventions would have been obvious to one of ordinary skill in the art. The court in *General Foods Corp. v. Studiengesellschaft Kohle GmbH*, (972 F.2d 1272, 1279, 23 USPQ2d 1839, 1844 (Fed. Cir.. 1992)) has stated that "the determining factor in deciding whether or not, there is double patenting is the existence vel non of *patentable differences* between two sets of claims." "It is important to bear in mind that comparison can be made only with what invention is claimed in the earlier patent, paying careful attention to the rules of claim interpretation to determine what invention a claim defines and not looking to the claim for anything that happens to be mentioned in it as though it were a prior art reference." (*General Foods*, 972 F.2d at 1280.). In General Foods the court found that an issued claim that included several elements did not render obvious, under the judicially created doctrine of double patenting, a claim to a single element within the combination. Specifically the court stated that "[a]nything less than a process with all 9 steps is not what is claimed, and is, therefore, not patented. Claims must be read as a whole in analyzing a claim of double patenting" and "this concept violates the fundamental rule of claim construction, that what is claimed is what is *defined by the claim taken as a whole*, every claim limitation (here each step) being material." (*General Foods*, 972 F.2d at 1278-80.)

In the instant application the claimed invention is directed to methods for mediating RNA interference of an mRNA in a cell *in vitro* by introducing in to the cell double stranded RNAs of 21-23 nucleotides in length that mediate RNA interference of an mRNA wherein the dsRNA is in the form of two separate strands which are not covalently linked.

The claims of the instant application encompass the use of a genus of double stranded RNA having certain structural features, *e.g.*, length, complementarity with the target mRNA, two separate strands not covalently linked, coupled with the functional ability to mediate RNAi. The claims of US 10/832257 are also directed to methods of mediating RNA interference using double stranded

RNAs, however, the claims of US 10/832257 feature methods of use of a different genera of RNAs as compared to the genus of double stranded RNAs useful according to Applicants' claimed methods. In particular, the claims of US 10/832257 feature methods of using double stranded RNAs wherein each strand is 19-23 or 19-25 nucleotides in length, at least one strand having a 3'overhang of 1-5 or 1-3 nucleotides. The claims of the instant invention are silent as to the occurrence of a 3' overhang, whereas the claims of US 10/832257 feature methods of using double stranded RNAs which must include a 3' overhang of 1-3 or 1-5 nucleotides in length and do not include the limitation that the RNA is in the form of two separate strands which are not covalently linked.

The claims of US 10/433050 and 11/142866 are not directed to methods for mediating RNA interference. Rather, the claims of US 10/433050 are directed to isolated dsRNA and the claims of US 11/142866 are directed to methods of stabilizing dsRNA. A method of mediating RNAi would not have been obvious in view of isolated dsRNA and methods for stabilizing dsRNA. Both claim sets also require the use of 3' overhangs and do not include the limitation that the RNA is in the form of two separate strands which are not covalently linked.

The fact that there may be overlap between the claims does not establish that the pending claims are obvious variants of the claims of US 10/832257, 10/433050 and 11/142866. At the time of the instant application, March 2000, one of skill in the art would not have modified the methods claimed in US 10/832257, 10/433050 and 11/142866 to arrive at the methods claimed in the instant application. In particular, the skilled artisan would not have been motivated, based on the teachings of the US 10/832257, 10/433050 and 11/142866 claims directed to double stranded RNAs having '3 overhangs of 1-3 or 1-5 nucleotides, to arrive at the claimed invention featuring methods of using the claimed genus of double stranded RNAs in the form of two separate strands which are not covalently linked because at the time of the invention there was no teaching that such molecules having the recited structural features were a desirable genus of RNAi-mediating molecules. By relying on a portion of the claim rather than all of the elements the examiner is using the claim as though it were the prior art rather than determining what the claim discloses as a whole and determining if they are the same invention.

Accordingly, Applicants request withdrawal of the rejection in view of US Patent Applications No. 10/832,257; 11/142,866 and 10/433,050.

Claims 17, 20-23, 76 and 80-85 have been provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 76-78, 81, 86-88, 91, 108, 110, 112, 115-120 and 124-177 of copending Application No. 09/821,832. The Office has maintained the rejection over US 09/821,832 because the instant application is the later filed application. Applicants disagree.

The Office should withdraw the provisional double patenting rejection in the instant application and allow the claims to proceed to allowance. A terminal disclaimer is not required over claims which have the same effective filing date as the instant claims and which have not yet been indicated to be allowable. The Office has indicated that "a common issue/filing date alone does not avoid the potential problems of dual ownership by a common assignee." Although a common filing date is not sufficient in itself to negate the need for a terminal disclaimer, the fact that two patent applications have a common priority date is an important point in determining whether a terminal disclaimer is required in one case versus the other case. Two applications having the same effective filing date are considered to have been filed on the same day. The two applications have the same expiration date. In deciding which case to require the filing of a terminal disclaimer between two applications having the same filing date and both being directed to the base invention, the Office should consider only which claims are allowable first.

MPEP 804(I)(B)(1) states:

> If "provisional" ODP rejections in two applications are the only rejections remaining in those applications, the examiner should withdraw the ODP rejection in the earlier filed application thereby permitting that application to issue without need of a terminal disclaimer. A terminal disclaimer must be required in the later-filed application before the ODP rejection can be withdrawn and the application permitted to issue. If both applications are filed on the same day, the examiner should determine which application claims the base invention and which application claims the improvement (added limitations). The ODP rejection in the base application can be withdrawn without a terminal disclaimer, while the ODP rejection in the improvement application cannot be withdrawn without a terminal disclaimer.

MPEP 804(I)(B)(1) states that the Office should withdraw an ODP rejection in the application with the earlier filing date and if the applications have the same filing date, the Office should determine which application claims the base invention and withdraw the rejection in that application. The instant application and US 09/821,832 have the same effective filing date. Both sets of claims derive priority to the same provisional patent application filed March 30, 2000. Both sets of claims are directed to the base invention rather than an improvement. MPEP 706.02 makes clear that patent applications claiming priority through 35 USC 120 have the same effective filing date. MPEP 706.02 (VI) states:

> The effective filing date of a U.S. application may be determined as follows:
>
> (A) If the application is a continuation or divisional of one or more earlier U.S. applications or international applications and if the requirements of 35 USC 120 and 365(c), respectively, have been satisfied, the effective filing date is the same as the earliest filing date in the line of continuation or divisional applications.

Once the claims are otherwise in condition for allowance, the ODP rejection should be withdrawn in the instant application. If the claims of US 09/821,832 have not yet been allowed, the Office can request that a Terminal Disclaimer be filed in that application, if appropriate.

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, the Director is hereby authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 23/2825, under Docket No. W0571.70010US03.

Dated: June 18, 2009

Respectfully submitted,

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Differential discrimination of DNA polymerases for variants of the non-standard nucleobase pair between xanthosine and 2,4-diaminopyrimidine, two components of an expanded genetic alphabet

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ABSTRACT

Mammalian DNA polymerases α and ε, the Klenow fragment of *Escherichia coli* DNA polymerase I and HIV-1 reverse transcriptase (RT) were examined for their ability to incorporate components of an expanded genetic alphabet in different forms. Experiments were performed with templates containing 2′-deoxyxanthosine (dX) or 2′-deoxy-7-deazaxanthosine (c^7dX), both able to adopt a hydrogen bonding acceptor–donor–acceptor pattern on a purine nucleus (puADA). Thus these heterocycles are able to form a non-standard nucleobase pair with 2,4-diaminopyrimidine (pyDAD) that fits the Watson–Crick geometry, but is joined by a non-standard hydrogen bonding pattern. HIV-1 RT incorporated d(pyDAD)TP opposite dX with a high efficiency that was largely independent of pH. Specific incorporation opposite c^7dX was significantly lower and also independent of pH. Mammalian DNA polymerases α and ε from calf thymus and the Klenow fragment from *E.coli* DNA polymerase I failed to incorporate d(pyDAD)TP opposite c^7dX.

INTRODUCTION

Nucleobases in oligonucleotide strands form Watson–Crick base pairs following two rules of complementarity: (i) a large purine from one strand pairs with a small pyrimidine from the other; (ii) hydrogen bond donors (NH groups) from one base are matched with hydrogen bond acceptors (lone pairs of electrons on oxygen or nitrogen) from the other. In DNA, for example, cytosine, implementing a hydrogen bond donor–acceptor–acceptor pattern (pyDAA), pairs as the small component with guanine, a large component implementing the hydrogen bond acceptor–donor–donor pattern (puADD).

Some time ago we pointed out that standard nucleobases exploit only part of the potential of the Watson–Crick formalism (1). When fully exploited the Watson–Crick formalism permits 12 independently replicatable nucleobases joined in six base pairs by mutually independent hydrogen bonding patterns (Fig. 1). Previous work in these and other laboratories has yielded implementations of all six hydrogen bonding patterns (2–6). Further, individual RNA and DNA polymerases have been found that catalyze template-directed incorporation of several non-standard base pairs into duplex DNA (7–9). However, DNA polymerases involved in DNA transactions in mammals have so far rejected non-standard base pairs.

As non-standard nucleobases are accepted by at least some polymerases, these bases must be intrinsically able to form Watson–Crick base pairs during a polymerization reaction, just as they contribute to the overall duplex stability in complementary oligonucleotide strands (4,10). The polymerases that do not accept non-standard nucleobases must, therefore, recognize some structural feature of the non-standard nucleobases incidental to their ability to form a competent Watson–Crick structure.

Recent studies in these laboratories have focused on the non-standard base pair between xanthosine (trivially designated X), which presents a hydrogen bond 'acceptor–donor–acceptor' (puADA) pattern to the complementary non-standard base 2,4-diaminopyrimidine (presenting a 'donor–acceptor–donor' hydrogen bonding pattern), designated here pyDAD (9). We have shown that the Klenow fragment of DNA polymerase I accepts dX as a nucleoside triphosphate opposite d(pyDAD) in the template, while human immunodeficiency virus type I (HIV-1) reverse transcriptase (RT) accepts dX and d(pyDAD) both in the template and as a triphosphate. The efficiency of incorporation of this non-standard base pair was generally lower, however, compared with the incorporation of standard nucleobases. Further, with a pK_a of 5.7 when free in solution (11), xanthosine is far more acidic as a heterocycle than standard nucleobases. While its pK_a should be higher when incorporated into an oligonucleotide, this acidity might also prove to be a problematical aspect of the non-standard nucleobase.

To explore this idea 2′-deoxy-*N*1-methyloxoformycin B, trivially designated dπ, was examined. The nucleoside bearing this heterocycle also presents a pu(ADA) hydrogen bonding

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EXHIBIT 1

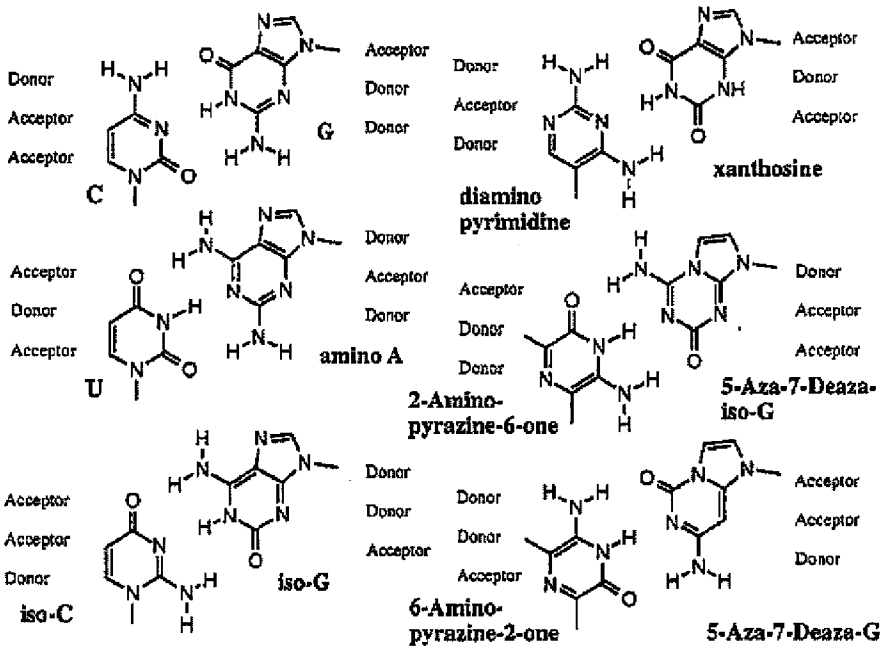

Figure 1. Six base pairs that meet the constraints imposed by the Watson–Crick base pairing geometry.

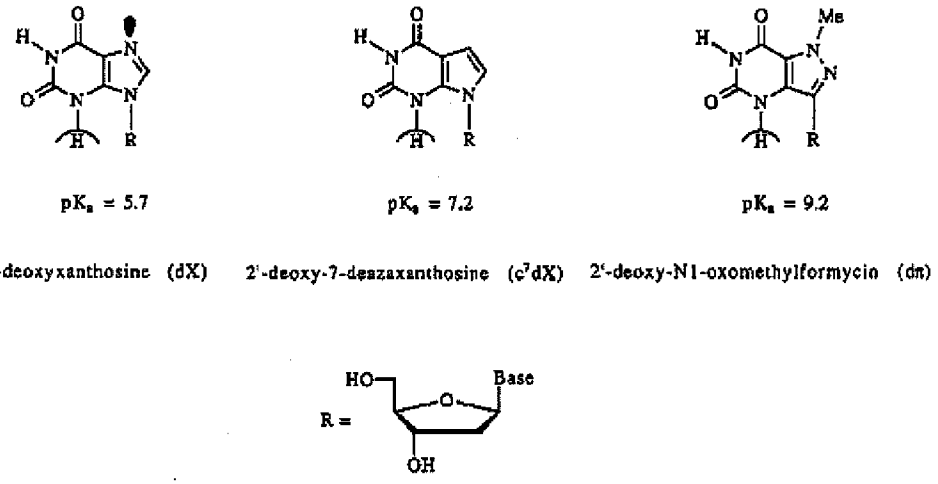

Figure 2. Three implementations of the 'acceptor–donor–acceptor' hydrogen bond pattern on variants of xanthosine.

pattern (Fig. 2), but has a pK_a of 9.2 (12). No polymerase was found to be able to synthesize a base pair between dπ and d(pyDAD), either when dπ was in the template or when it was presented as the triphosphate (9). However, dπ has other differences that distinguish it from dX. First, it is a C-glycoside, the heterocyclic base being joined to the 2′-deoxysugar by a carbon–carbon bond. This was known to exert a small but significant (~3-fold) effect on incorporation with some polymerases (13). Further, dπ is modified at the N-7 position, replacing the lone pair of electrons in xanthosine at this position by a methyl group. Each of these differences could also account for the ability of HIV-1 RT to accept dX but not dπ.

The 2′-deoxy-7-deazaxanthosine (c^{7}dX) (Fig. 2) nucleoside also implements the pu(ADA) hydrogen bonding pattern found in dX and dπ. Like dπ, c^{7}dX is missing the lone pair of electrons at N-7 through replacement of N-7 with a CH group, but does not have a bulky methyl group at this position. Further, the nucleobase has a pK_a value of 7.2 (14), presumably corresponding to deprotonation at N-3. We report here the enzymology of the py(DAD)·c^{7}dX base pair.

MATERIALS AND METHODS

Synthesis of non-standard nucleobases

2,4-Diamino-5-(β-D-ribofuranosyl)pyrimidine (pyDAD) was synthesized using the route of Chu *et al.* (3). This compound was converted to the 2′-deoxygenated nucleoside analog as described by Piccirilli *et al.* (4). The triphosphate d(pyDAD)TP was synthesized by a published procedure (15). 5′-Dimethoxytrityl-2′-deoxyxanthosine with both heterocyclic ring oxygens protected as *p*-nitrophenylethyl ethers was prepared by the procedure of Van Aerschot *et al.* (16) and converted to the phosphoramidite following a standard method (17). 2′-Deoxy-7-deazaxanthosine (c^7dX) was synthesized as 7-deaza-2′-deoxy(4,4′-dimethoxytrityl)xanthosine-3′-H-phosphonate as recently described (18). Standard dNTPs were from Pharmacia.

Oligonucleotides

The oligonucleotide bearing 2′-deoxyxanthosine was prepared by solid phase synthesis (Applied Biosystems) from the β-cyanoethyl-protected phosphoramidite, purified by the trityl-on procedure, deprotected and purified again by HPLC (19). The oligonucleotide bearing c^7dX was synthesized by Dr L.Arnold (Czech Academy of Chemistry and Biochemistry, Prague) using H-phosphonate technology.

The primer (5′-GCATGGATCCCACTGCACTCCAGGG-3′) was synthesized by Microsynth (Windisch, Switzerland) and purified by PAGE. Labelling of the primer at the 5′-end with Redivue [γ-^{32}P]ATP (Amersham) was performed using T4 polynucleotide kinase (Life Technologies).

Nucleic acid substrates

The primer was annealed with a template (5′-ACCCCqCCCCCC-CTGGAGTGCAGTGGGATCCATGC-3′), where q is either dX or c^7dX, in 500 µl total buffer containing 50 pmol template and 15 pmol labelled primer in 1.8 mM Tris–HCl, pH 7.0, 0.5 mM $MgCl_2$, 23 mM NaCl by heating the mixture at 85°C for 15 min followed by subsequent slow cooling to room temperature over a period of 1 h.

DNA polymerases

HIV-1 RT, overexpressed using the plasmid pJS3.7 in *Escherichia coli*, was purified by a published procedure (20). Calf thymus DNA polymerases α and ε were purified according to the methods of Podust *et al.* (21) and Weiser *et al.* (22) respectively. Enzymatic activity was determined as described in these references. The Klenow fragment of DNA polymerase I was from Boehringer Mannheim.

Assays to detect incorporation of the bases

Incorporation of a non-standard base opposite the complementary non-standard base was performed in a total volume of 25 µl using 0.15 pmol labelled and annealed primer and all required dNTPs, at a final concentration of 5 µM each. Reaction buffers contain the following: for HIV-1 RT, 50 mM Tris–HCl, pH 7.2 (unless otherwise stated), 5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 0.5 mM EDTA; for DNA polymerases α and ε from calf thymus, 50 mM Tris–HCl, pH 6.5, 1 mM DTT, 0.25 mg/ml BSA; for Klenow fragment of DNA polymerase I, 50 mM Tris–HCl, pH 7.5, 1 mM DTT, 0.1 mg/ml BSA. The amount of enzyme used was 0.1 U Klenow fragment and HIV-1 RT, 0.11 U DNA polymerase α and 0.04 U DNA polymerase ε. The reactions were started by adding the enzyme and incubated for 15 min at 37°C and finally quenched by adding 5 µl of a mixture of stop/loading dye (New England Biolabs), which contains 0.3% xylene cyanol, 0.3% bromphenol blue and 0.37% Na EDTA, pH 8.0. The samples were heated (20 min, 95°C) and aliquots (5 µl) were loaded onto a 17% polyacrylamide gel containing 7 M urea. Following electrophoresis (constant power 25 W) the gels were fixed (12% MeOH, 10% HOAc, diluted with water), dried and autoradiographed. Radioactivity was quantified using a PhosphorImager (Molecular Dynamics), with 3 h exposures and the ImageQuant program from Molecular Dynamics. To determine the amount of specific formation of the non-standard base pairs the amount of full-length product was quantified, divided by the total amount of radioactivity in the lane and expressed as a percentage. To correct for non-specific misincorporation of standard nucleobases opposite the non-standard nucleotides the amount of misincorporation of natural dNTPs, determined in a control experiment, was subtracted.

RESULTS

No evidence could be obtained for incorporation of d(pyDAD)TP opposite c^7dX in a template when Klenow fragment of DNA polymerase I from *E.coli* was incubated at pH 7.5. Oligonucleotide products indicating extension of the primer past the non-standard base were found both in the presence and absence of d(pyDAD)TP. It is possible that the Klenow fragment misincorporates dGTP opposite c^7dX (Fig. 3, lanes 5–7). However, the principal product is shorter than the full-length product by one base. Why this $n-1$ product is formed is not known. It may arise from the DNA polymerase skipping over the non-standard nucleobase or may be a response of the DNA polymerase to a mismatch in the template–primer complex. Similar production of $n-1$ product has been observed with other unsuccessful fill-in experiments using Klenow fragment (4, 9). In any case, a quantitative analysis using a PhosphorImager shows that at most 1% of the longest product is derived from specific incorporation of d(pyDAD)TP opposite c^7dX in the template under these conditions.

Similarly, neither calf thymus DNA polymerases α nor ε incorporated d(pyDAD)TP opposite c^7dX in a template at pH 6.5. Less misincorporation was observed with these DNA polymerases (Fig. 3, lanes 8–13), consistent with the overall higher fidelity of these polymerases in general (9). The quantitative analysis yields ~1.5% specific formation of the non-standard base pair for DNA polymerase α and ~1.9% for DNA polymerase ε, within the experimental error. These mammalian DNA polymerases also yielded full-length product missing the final base.

When HIV-1 RT was incubated with (pyDAD)TP and a template containing c^7dX and d(pyDAD)TP at pH 7.2 (Fig. 3, lanes 2–4) full-length product was observed in excess of that formed when d(pyDAD)TP was omitted. This suggested that (pyDAD)TP was successfully incorporated opposite c^7dX. However, the efficiency of incorporation of d(pyDAD)TP was much lower than that observed with an analogous template containing dX instead of c^7dX (Fig. 4a). Furthermore, evidence for misincorporation of dGTP opposite c^7dX could be seen. Quantitative analysis shows that only ~8% of the amount of

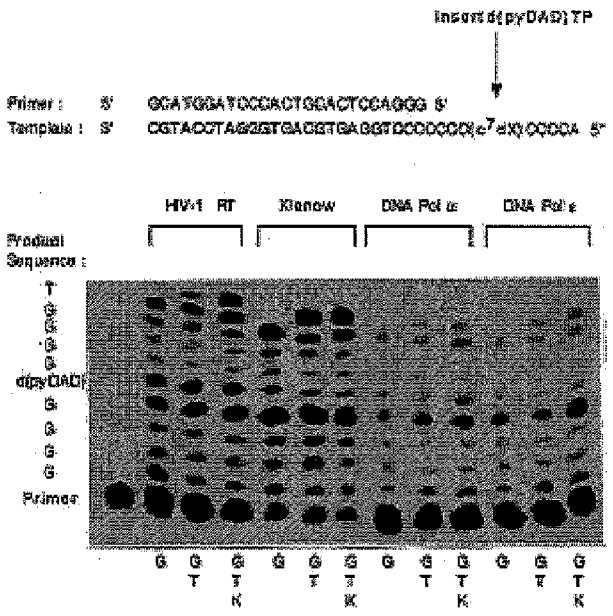

Figure 3. Primer extension by mutants of HIV-1 RT, Klenow fragment and mammalian DNA polymerases α and ε. Deoxynucleoside triphosphates present are indicated below, where K stands for d(pyDAD)TP. Deoxynucleoside triphosphates (5 μM) were incubated at 37°C for 15 min with 0.15 pmol primer–template complex containing the c^7dX nucleobase in the template and 0.1 U HIV-1 RT and Klenow fragment, 0.11 U DNA polymerase α and 0.04 U DNA polymerase ε in a final volume of 25 μl.

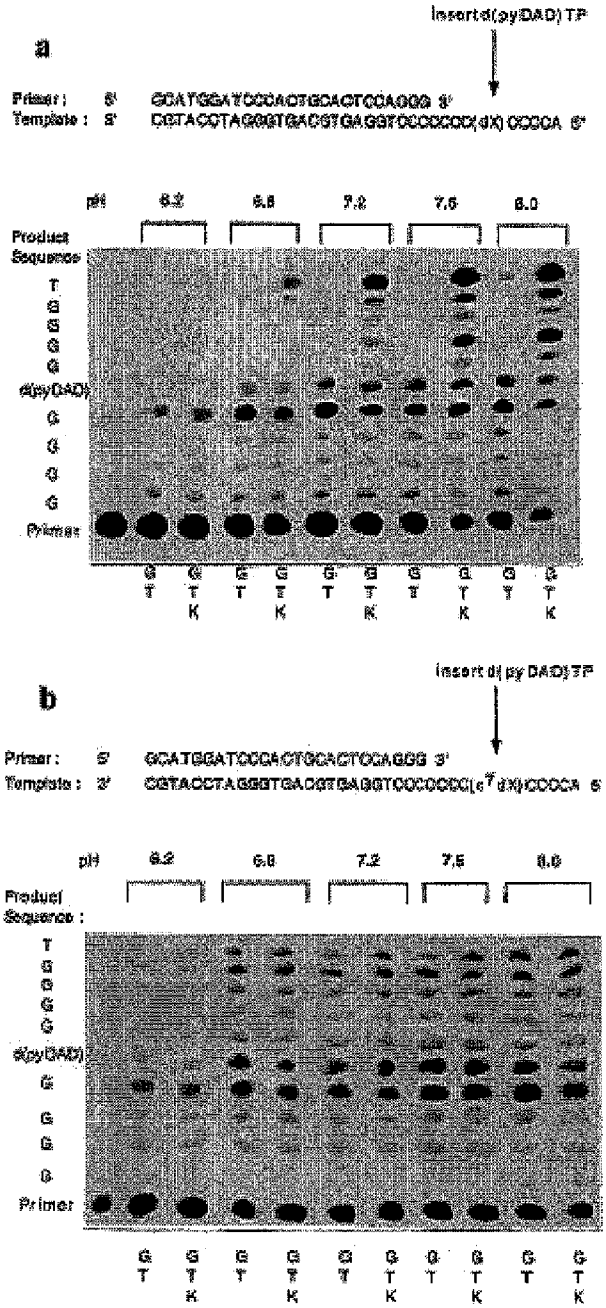

Figure 4. pH-dependent primer extension by HIV-1 RT. Deoxynucleoside triphosphates present are indicated below, where K stands for d(pyDAD)TP. Deoxynucleoside triphosphates (5 μM) were incubated at 37°C for 15 min with 0.15 pmol primer–template complex containing the (**a**) dX and (**b**) c^7dX nucleobase in the template and 0.1 U HIV-1 RT in a final volume of 25 μl.

full-length product derives from the incorporation of d(pyDAD)TP opposite c^7dX in the template.

Templates containing dX successfully direct incorporation of d(pyDAD)TP at pH 7.2 when HIV-1 RT is the catalyst. Remarkably, very little (if any) misincorporation is observed opposite dX when HIV-1 RT is used (Fig. 4a). The pH dependence of this incorporation was then studied (Fig. 4a) with a template containing dX and d(pyDAD)TP to be incorporated. A quantitative analysis shows that the amount of full-length product increases by ~3-fold with increasing pH over the range 6.8–8.0 (Fig. 4a). The maximum amount of full-length product formed under these conditions was ~30% at pH 7.5 and then drops to ~26% at pH 8.0. However, virtually all of the increase in the synthesis of full-length product is due to increased activity of the enzyme (~3-fold) at higher pH. Slight misincorporation of standard nucleobases opposite dX was observed, but only at pH 8.0.

Incorporation of d(pyDAD)TP opposite c^7dX in the template showed only slight pH dependency. With c^7dX the increase in enzymatic activity over the pH range 6.2–8.0 is only about a factor of two. Quantitative analysis using a PhosphorImager shows for this pH-dependent study that the amount of full-length product formed by specific incorporation of d(pyDAD)TP opposite c^7dX in a template reaches a maximum at pH 7.2 of ~5.5% under these conditions and then drops to a value of ~1.5% at pH 8.0.

Further pH dependence studies were performed at pH values of 8.0–9.5. Experiments with dX in the template show that the amount of full-length product due to specific incorporation of d(pyDAD)TP decreases with increasing pH. However, the amount of full-length product due to misincorporation of standard nucleobases increases with increasing pH. At pH 9.5 full-length product derives only from misincorporation (data not shown). Similiar results were seen when c^7dX was in the template. The amount of full-length product decreases with increasing pH and

no specific incorporation of d(pyDAD)TP opposite c^7dX was observed over this pH range (data not shown). Needless to say, HIV-1 RT has low catalytic activity under these high pH conditions.

DISCUSSION

The standard model of nucleic acid structure, proposed in its original form over four decades ago by Watson and Crick (23), invokes the stacking of hydrophobic nucleobases as a central determinant of the stability of the double helix. In its simplest form this model suggests that the less hydrophobic a nucleobase, the less likely it is to be accepted into a duplex structure by a DNA polymerase. Naively, this implies that given the choice between a more acidic nucleobase (in this example dX) and a less acidic nucleobase (c^7dX), both meeting the minimum hydrogen bonding requirements, the latter would be more easily accepted than the former.

This is not the case. A variety of polymerases accept c^7dX as a complement for (pyDAD)TP more poorly than dX; several do not accept it at all. Further, incorporation of (pyDAD)TP opposite dX in a template is largely independent of pH over the range 6.2–9.5. This pH range is expected to span the pK_a of dX in a template, as the pK_a of dX free in solution (5.7) is expected to be increased by 2 to 3 pK_a units when incorporated into a polyanionic oligonucleotide, according to the observed shift with 7-methyl-2′-deoxyguanine and guanylic acid when embedded in a DNA oligonucleotide (24,25). As the pK_a of the nucleobase can be further perturbed in the active site of a DNA polymerase, the ionization state of dX in a template at the instant when the molecular recognition event occurs is not easily known. However, it is clear that the intrinsic acidicity of dX does not present an obvious impediment to its serving as a partner in a Watson–Crick base pair.

Why is c^7dX accepted less efficiently (or not at all) than its analog dX? Three explanations might be considered.

(i) Substitution of N-7 in dX by a CH group in c^7dX might create structural perturbations that might be invoked to explain this discrimination against c^7dX. For example, the conformation of the base or the sugar might be influenced by this substitution.

(ii) Alternatively, the DNA polymerase might actually recognize the deprotonated form of dX, a form that cannot be attained by c^7dX due to its higher pK_a.

(iii) The DNA polymerase might itself interact with N-7 in a way that causes it to reject c^7dX as foreign. This proposal suggests that the DNA polymerase is 'scanning' the major groove of duplex DNA.

Each of these possibilities raises interesting questions concerning the event by which DNA polymerases recognize base pairs. Explanation (i) is problematical, because structural differences induced by the N-7 substitution are expected to be subtle. Further, HIV-1 RT seems to be largely indifferent to subtle structural features of the nucleobase. For example, it accepts both DNA and RNA as template, which have quite different conformations.

Explanation (ii) is problematical considering the fact that incorporation of dX is essentially pH independent. If the DNA polymerase indeed prefers a deprotonated form of the nucleobase over the protonated form, one might expect the efficiency of incorporation of dX to increase with increasing pH. This is not the case. Further, if the relative pK_a values of dX and c^7dX in the template are the same as the relative pK_a values of dX and c^7dX free in solution and if the only impact of the substitution at position 7 is the shift in pK_a then c^7dX at pH 8.5 should behave the same as dX at pH 7.0, but it does not.

The remaining possibility is that the DNA polymerase is itself examining structural features of the nucleobases, presumably in the major and minor grooves, to discard 'unnatural' structures. At one level this proposal is reasonable. To enforce a Watson–Crick geometry the DNA polymerase must interact in some way with the nucleobases, in either the major or minor groove. This interaction presumes a direct contact between functionality on the bases and functionality in the protein. This proposal is problematical, however, as different nucleobases present different functionality in these grooves and DNA polymerase should have no intrinsic preference for one nucleobase over another, once the nucleobase has been accepted by the template.

Thus DNA polymerases, if they are to interact with the nucleobases to enforce a Watson–Crick geometry, must do so by identifying features in the grooves of duplex oligonucleotides that are constant for all four nucleobases. One such feature exists. In the minor groove the lone pair of electrons on N-3 of both purines approximately overlap in space the lone pair of electrons presented by the 2-position carbonyl oxygens of both thymine and cytosine. Thus it is conceivable that a DNA polymerase might present a hydrogen bond donor to this lone pair in all four bases, allowing it to control the geometry of the incoming nucleobase without having a preference for one over the other. Several years ago Steitz noted that such minor groove 'scanning' might be used by DNA polymerases to improve their fidelity (26). Furthermore, the recently published crystal structure of mammalian DNA polymerase β co-crystallized with template, primer and a triphosphate analog identified three amino acid residues that make contacts with these lone pairs (27).

The results reported here are inconsistent with the scanning proposal in its broadest form, as a lone pair of electrons at position O-2 in pyrimidines is not an absolute requirement for recognition by DNA polymerases. The pyDAD nucleobase lacks the exoxyclic oxygen and would not be accepted by any polymerase if the lone pair were an absolute specificity determinant. As we have shown here and elsewhere (9), pyDAD is accepted by many polymerases, either in the template or as a triphosphate. Further, in its protonated form dX also lacks the lone pair of electrons at N-3 and yet is also accepted by DNA polymerases, although the possibility remains that the polymerase is accepting the N-3 deprotonated form of the nucleobase, which carries the lone pair.

Explanation (iii) requires, however, a new type of scanning, in the major groove. This scanning is also problematical, as no functional group is consistently presented to the major groove by the standard nucleobases. For example, thymine presents a hydrophobic methyl group to one region of the major groove, cytosine presents a hydrogen atom and both purines present a hydrogen bond acceptor, a lone pair of electrons on N-7. These functionalities are different and it is difficult to imagine a DNA polymerase making a contact with this region of the major groove without causing it to favor one of the standard bases over any other in a way that would diminish faithful reproduction of information in the template.

The disfavoring of c^7dX is more perplexing in the light of former results showing that Klenow fragment and Taq DNA polymerase both accept 7-deaza-dGTP (28,29), as well as dGTP substituted at the N-7 position with either a methyl group or cyanoborane (24,30). Because Klenow fragment rejects

d(pyDAD) as the triphosphate, both opposite dX and c^7dX, its rejection of c^7dX is more difficult to interpret. Nevertheless, Klenow fragment does not seem to require a lone pair of electrons on N-7 in the major groove for all purines.

These data suggest a paradox in the 'model' for the selectivity of polymerases. The selectivity of individual polymerases (such as Klenow fragment) with respect to variants of non-standard nucleobases seems to be unrelated to their selectivity with respect to analogous variants of the standard nucleobases. There is no simple structural explanation for this fact. Further, even though crystal structures compellingly argue that all polymerases are related by common ancestry (31), it is clear that the details of the molecular recognition process diverge greatly with their sequences. There is not likely to be a general model describing DNA polymerase specificity generally; each polymerase will need to be described individually, with more work both in solution with non-standard nucleobases and other nucleotide analogs and in the crystal.

ACKNOWLEDGEMENTS

We thank Drs J.Opitz and A.Roughton for helpful discussions. We are indebted to Dr L.Arnold from the Czech Academy of Organic Chemistry and Biochemistry in Prague for oligonucleotide synthesis. MJL was supported by a scholarship from the German Academic Exchange Service (DAAD) in the program HSP II/AUFE and MH and UH were supported by the Swiss National Science Foundation (grants 3135-36713.92 and 31.37146.93).

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Electronic Patent Application Fee Transmittal

Application Number:	10255568
Filing Date:	26-Sep-2002
Title of Invention:	RNA sequence-specific mediators of RNA interference
First Named Inventor/Applicant Name:	Thomas Tuschl
Filer:	Helen C. Lockhart/Janet D'Annunzio-Ellis
Attorney Docket Number:	0399.2008-013

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1110	1110

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**1110**

Electronic Acknowledgement Receipt

EFS ID:	5542867
Application Number:	10255568
International Application Number:	
Confirmation Number:	2920
Title of Invention:	RNA sequence-specific mediators of RNA interference
First Named Inventor/Applicant Name:	Thomas Tuschl
Customer Number:	23628
Filer:	Helen C. Lockhart/Janet D'Annunzio-Ellis
Filer Authorized By:	Helen C. Lockhart
Attorney Docket Number:	0399.2008-013
Receipt Date:	18-JUN-2009
Filing Date:	26-SEP-2002
Time Stamp:	14:57:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1110
RAM confirmation Number	1087
Deposit Account	232825
Authorized User	LOCKHART,HELEN C

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	W057170010US03-TRN-HCL.pdf	32224 74ccfffa3230fc6c7903ffb5002e824fab5fd4c0	no	1
Warnings:					
Information:					
2	Fee Worksheet (PTO-875)	W057170010US03-FEE-HCL.pdf	46904 bac14b25a7613156e880f4bbfacf022bee51900d	no	1
Warnings:					
Information:					
3	Extension of Time	W057170010US03-EXT-HCL.pdf	36781 deb0ee5d6acdbf5d81f85c84206dff2703688d60	no	1
Warnings:					
Information:					
4		W057170010US03-AMN-HCL.pdf	978703 01ee5f02aaf7c8837669e1c0c6db0f660fe89f2d	yes	20

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	5
Applicant Arguments/Remarks Made in an Amendment	6	20

Warnings:					
Information:					
5	Fee Worksheet (PTO-875)	fee-info.pdf	30189 d5a94c2568610cedfc6844a97ee801c674cdfbe0	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	1124801	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/21 (01-09)
Approved for use through 02/28/2009. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	23

Application Number	10/255,568-Conf. #2920
Filing Date	September 26, 2002
First Named Inventor	Thomas Tuschl
Art Unit	1635
Examiner Name	L. V. Wollenberger
Attorney Docket Number	W0571.70010US03

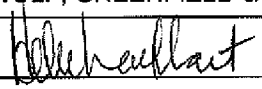
ENCLOSURES (*Check all that apply*)

- [x] Fee Transmittal Form
- [ ] Fee Attached
- [x] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please identify below):

Transmittal Letter
Exhibit 1 - Copy of Lutz et al., Nucleic Acids Research 24(7):1308-1313 (1996)

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature			
Printed name	Helen C. Lockhart		
Date	June 18, 2009	Reg. No.	39,248

Certificate of Electronic Filing Under 37 CFR 1.8

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Dated: June 18, 2009 Signature:  (Janet D'Annunzio-Ellis)

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Approved for use through 06/30/2010. OMB 0651-0032
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2009

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 1,110.00

Complete if Known	
Application Number	10/255,568-Conf. #2920
Filing Date	September 26, 2002
First Named Inventor	Thomas Tuschl
Examiner Name	L. V. Wollenberger
Art Unit	1635
Attorney Docket No.	W0571.70010US03

METHOD OF PAYMENT (check all that apply)

☐ Check ☒ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☐ Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fees Paid ($)
Utility	330	165	540	270	220	110	
Design	220	110	100	50	140	70	
Plant	220	110	330	165	170	85	
Reissue	330	165	540	270	650	325	
Provisional	220	110	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	52	26
Each independent claim over 3 (including Reissues)	220	110
Multiple dependent claims	390	195

Total Claims		Extra Claims	Fee ($)		Fee Paid ($)
19	- 75 =	0	x 0	=	0

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		Extra Claims	Fee ($)		Fee Paid ($)
3	- 23 =	0	x 0	=	0

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
0	0

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof		Fee ($)		Fee Paid ($)
	- 100 =		/50 =		(round up to a whole number) x		=	

4. OTHER FEE(S) Fees Paid ($)

Non-English Specification, $130 fee (no small entity discount)

Other (e.g., late filing surcharge): 1253 Extension for response within third month 1,110.00

SUBMITTED BY

Signature	Helen Lockhart	Registration No. (Attorney/Agent)	39,248	Telephone	617.646.8000
Name (Print/Type)	Helen C. Lockhart			Date	June 18, 2009

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Dated: June 18, 2009 Signature: Janet D'Annunzio-Ellis (Janet D'Annunzio-Ellis)

PTO/SB/22 (01-09)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2009 *(Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)*	Docket Number (Optional) W0571.70010US03
Application Number 10/255,568-Conf. #2920	Filed September 26, 2002
For RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE	
Art Unit 1635	Examiner L. V. Wollenberger

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	
[ ]	One month (37 CFR 1.17(a)(1))	$130	$65	$
[ ]	Two months (37 CFR 1.17(a)(2))	$490	$245	$
[X]	Three months (37 CFR 1.17(a)(3))	$1110	$555	$ 1,110.00
[ ]	Four months (37 CFR 1.17(a)(4))	$1730	$865	$
[ ]	Five months (37 CFR 1.17(a)(5))	$2350	$1175	$

[ ] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check in the amount of the fee is enclosed.

[X] Payment by credit card. ~~Form PTO-2038 is attached.~~

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 23/2825 .

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

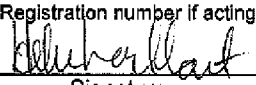
I am the

[ ] applicant/inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

[X] attorney or agent of record. Registration Number 39,248

[ ] attorney or agent under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34 _____



Signature — June 18, 2009 (Date)

Helen C. Lockhart (Typed or printed name) — 617.646.8000 (Telephone Number)

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[ ] Total of 1 forms are submitted.

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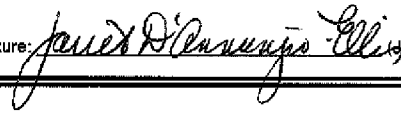
Dated: June 18, 2009 Signature:  (Janet D'Annunzio-Ellis)

EXHIBIT 3

Wolf Greenfield
SPECIALISTS IN INTELLECTUAL PROPERTY LAW

Helen C. Lockhart
hlockhart@wolfgreenfield.com
direct dial 617.646.8259

January 21, 2009

Monica Gerber
Intellectual Property Counsel
Whitehead Institute for Biomedical Research
Five Cambridge Center
Room 736
Cambridge, Massachusetts 02142

Re: U.S. Patent Application No.: 10/255,568
Entitled: RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE
Filing Date: September 26, 2002
Inventor(s): Thomas Tuschl et al.
Your Ref. No.: WHI00-06; MIT 8768
Our Ref. No.: W0571.70010US03

Dear Monica:

We received a non-final Office Action in connection with the above-identified patent application, a copy of which is enclosed. A response to this Office Action is due on March 18, 2009, although the time for responding may be extended until June 18, 2009 with the payment of successively-increasing monthly extension fees.

We attach hereto a draft response to the Office Action. Please review the draft response and provide us with your comments. We would like to collect comments on the draft by February 23, 2009. Please provide us with your comments by that date or let us know if you will need additional time.

As mentioned in the attached draft response, we have prepared Terminal Disclaimers to disclaim any terminal part of the statutory term of co-pending US 09/821832, if US 09/821832 issues. The instant application and US 09/821832 should have the same patent term. We will forward the Terminal Disclaimers and Statements 3.73(b) under separate cover to the co-owners of the patent application for review and signature.

Please note there is a continuing duty of disclosure as imposed under U.S. law to disclose all known prior art and other information that may be considered "material to patentability." We may need to provide such information to the U.S. Patent and Trademark Office. Therefore, please send us copies or complete identifications (including dates of publication) if you have not

Wolf Greenfield
SPECIALISTS IN INTELLECTUAL PROPERTY LAW

already done so. We will then file an Information Disclosure Statement in the U.S. Patent and Trademark Office.

In the meantime, if you have any questions or comments, please do not hesitate to contact us.

Very truly yours,

WOLF, GREENFIELD & SACKS, P.C.

Helen Lockhart /srl

Helen C. Lockhart

HCL/srl
Enclosures

cc:

Lauren Foster	Patricia A. Tuft	Brenda Herschbach Jarrell
K. Nicole Clouse	Robert B. Murray	Debra Milasincic
Thomas Tuschl	W. Weiss	Phillip D. Zamore
Martin Mullins	David P. Bartel	Phillip A. Sharp
Donna Ward	Shoji Takahashi	Carla Demaria
Adina Davis	Angie Bellanton	Zoran Zdraveski

DRAFT

Docket No.: W0571.70010US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Thomas Tuschl et al.
Serial No.:	10/255,568
Confirmation No.:	2920
Filed:	September 26, 2002
For:	RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE
Examiner:	L. V. Wollenberger
Art Unit:	1635

Certificate of Electronic Filing Under 37 CFR 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: _____ Signature: _____ (Sharon R. Lloyd)

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

INTRODUCTORY COMMENTS

In response to the Office Action mailed December 18, 2008, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Applicant has submitted a new complete claim set showing marked up claims with insertions indicated by underlining and deletions indicated by strikethroughs or double bracketing.

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1-16. (Canceled)

17. (Previously Presented) A method of mediating RNA interference of an mRNA in a cell in vitro comprising:

(a) introducing into the cell isolated double stranded RNA of from about 21 nucleotides to 23 nucleotides in length, wherein the double stranded RNA is in the form of two separate strands which are not covalently linked and has sequence correspondence to the mRNA and wherein the double stranded RNA mediates RNA interference by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within the region of sequence correspondence with the double stranded RNA, and wherein the mRNA is mammalian cellular mRNA;

(b) maintaining the cell produced in (a) under conditions such that degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA in the cell.

18. (Canceled)

19. (Canceled)

20. (Currently Amended) A method of mediating RNA interference of an mRNA in a cell in vitro, comprising:

(a) combining isolated double-stranded RNA, wherein the strands are not covalently linked, that corresponds to a sequence of the mRNA with a soluble extract that mediates RNA interference, thereby producing a combination, wherein the soluble extract is derived from syncytial blastoderm Drosophila embryos;

(b) maintaining the combination produced in (a) under conditions such that the double-stranded RNA is processed to double stranded RNA of from about 21 to about 23 nucleotides, thereby producing double stranded RNA of from about 21 to about 23 nucleotides;

(c) isolating double stranded RNA of from about 21 to about 23 nucleotides produced in (b);

(d) introducing double stranded RNA isolated in (c) into the cell; and

(e) maintaining the cell produced in (d) under conditions such that degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA in the cell.

21. (Canceled).

22. (Previously Presented) The method of Claim 20, wherein the double stranded RNA of (c) is isolated using gel electrophoresis.

23. (Previously Presented) A method of mediating RNA interference of an mRNA in a cell in vitro, comprising: (a) introducing into the cell isolated double stranded RNA of from about 21 nucleotides to 23 nucleotides in length, wherein the double stranded RNA is in the form of two separate strands which are not covalently linked and has sequence correspondence to the mRNA, wherein the double stranded RNA is chemically synthesized and wherein one or more nucleotides of the double stranded RNA are a non-naturally occurring nucleotide or deoxyribonucleotide or non-standard nucleotide, and wherein the double stranded RNA mediates RNA interference

by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within the region of sequence correspondence with the double stranded RNA, and wherein the mRNA is mammalian cellular mRNA and (b) maintaining the cell that contains the double stranded RNA such that degradation of the mRNA occurs, thereby mediating RNA interference of mRNA in the cell.

24.-75. (Canceled)

76. (Currently Amended) The method ~~of any one of claims 17, 20 and~~ of claim 23, wherein the mRNA is human mRNA.

77.-79. (Cancelled)

80. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is from 21 to 23 nucleotides in length.

81. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 21 nucleotides in length.

82. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 22 nucleotides in length.

83. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is 23 nucleotides in length.

84. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA is perfectly complementary to the mRNA.

85. (Currently Amended) The method ~~of any one of claims 17 and~~ of claim 23, wherein the double stranded RNA comprises a terminal 3' hydroxyl group.

86. (New) The method of claim 17, wherein the double stranded RNA is from 21 to 23 nucleotides in length.

87. (New) The method of claim 17, wherein the double stranded RNA is 21 nucleotides in length.

88. (New) The method of claim 17, wherein the double stranded RNA is 22 nucleotides in length.

89. (New) The method of claim 17, wherein the double stranded RNA is 23 nucleotides in length.

90. (New) The method of claim 17, wherein the double stranded RNA is perfectly complementary to the mRNA.

91. (New) The method of claim 17, wherein the double stranded RNA comprises a terminal 3' hydroxyl group.

92. (New) The method of claim 17, wherein the mRNA is human mRNA.

93. (New) The method of claim 20, wherein the mRNA is human mRNA.

REMARKS

Applicant respectfully requests reconsideration. Claims 17, 20-23, 76 and 80-85 were previously pending in this application. Claims 76 and 80-85 have been amended to limit the dependency to claim 23. New claims 86-93 have been added to add the subject matter of claims 76 and 80-85 as they depend from claims 17 or 20. Claim 20 is amended herein to add the limitation of former claim 21. Claim 21 is canceled. As a result, claims 17, 20, 22-23, 76, and 80-93 are pending for examination with claims 17, 20, and 23 being independent claims. No new matter has been added.

Interview Summary

Applicants thank Examiner Wollenberger for the courtesy of conducting a telephone interview on December 16, 2008. During the interview double patenting rejections of pending claims and written description rejection of claim 20 were discussed.

Withdrawn Rejections

The Examiner is thanked for the indication that the rejection of the claims under 35 USC 102(e) as being anticipated by Fire et al. is withdrawn. Applicant confirms the office position that "about 21 nucleotides to 23 nucleotides" in independent claims 17 and 23 is considered to embrace dsRNA shorter than 21 nucleotides but not longer than 23 nucleotides.

Priority

The Examiner has accorded an earliest effective filing date of January 31, 2001 to claims claims 23, 76, and 80-85. The other claims were accorded an earliest effective filing date of the provisional application filed March 30, 2000. Applicant disagrees with the assignment of the later priority date to claims 23, 76, and 80-85. US 60/193594 filed on March 30, 2000 describes the use of "altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides (e.g., analogs)." It was clear to the skilled artisan, at the time the priority document was filed, that the cited language referred to the use of non-standard

nucleotides. The ipsis verbis recitation of the term non-standard nucleotide is not required to provide an adequate written description of the invention. (See for instance MPEP 2163 II(a)3(a).)[1] At the time the application was filed it was understood in the art that standard nucleotides include A, C, T, G, and U. It was also understood that numerous other non-standard nucleotides exist and were useful in certain DNA and RNA based systems. For instance, US Patent 5,965,364 to Benner entitled "Method for selecting functional deoxyribonucleotide derivatives" which issued in 1999, before the priority date of the instant patent application, discloses the use of non-standard nucleotides. US Patent 5,965,364 describes the use of non-standard nucleotides or nucleobases in nucleic acid based libraries. It is pointed out in the patent that conventional nucleotides are "ribo or deoxyribo adenylic acid, guanylic acid, and cytidylic acid, and uridylic acid or thymidylic acid, referred to by convention as A, G, C, U and T." .

The term non-standard nucleotide is also described in US Patent No. 6,127,535, issued in 2000 and having a filing date in 1998. It is taught in US 6,127,535 that "The term 'nucleotide' as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. Nucleotides generally include a base, a sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; all hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as recently summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183."

Non-standard nucleotides are also described in the non-patent literature prior to Applicant's priority date. For instance, Lutz, et al, Differential discrimination of DNA polymerase for variants

[1] "If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient")."

of the non-standard nucleobase pair between xanthosine and 2,4-diaminopyrimidine, two components of an expanded genetic alphabet, Nucleic Acids Res. 1996 April 1; 24(7): 1308–1313, copy attached hereto, describes the use of non-standard nucleotides.

A person of ordinary skill would have understood, at the time the patent application was filed, that altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides (e.g., analogs) refers to non-standard nucleotides, i.e. nucleotides other than standard A, C, T, G, and U nucleotides. In view of the knowledge of the skilled artisan at the time of the invention one skilled in the art also would have been able to make and use dsRNA, as described in the instant specification, with non-standard nucleotides. Thus, US provisional patent application 60/193594 filed on March 30, 2000 provides an enabling and adequate written description for the term "non-standard nucleotide".

In order to clarify the record, Applicant has rewritten dependent claims 76 and 80-85 as they depend from claims 17 and 20 as separate dependent claims. The record is clear that new claims 86-93 are entitled to the earliest priority date associated with US 60/193594, March 30, 2000.

Rejection under 35 USC 112

Claim 20 has been rejected under 35 USC 112 first paragraph as failing to comply with the written description requirement. Although Applicant disagrees with the rejection, in order to advance prosecution, Applicant has amended claim 20 to incorporate the limitations of pending claim 21. Claim 21 has not been rejected. It is believed that the amendment is sufficient to overcome the rejection.

Double Patenting Rejection

Claims 17, 20, 23, 76 and 80-85 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 50-127 of copending Application No. 10/832,257.

Claims 17, 20-23, 76 and 80-85 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 48, 49, 51, 53-

57, 60-64, 67-73 and 75-125 of copending Application No. 10/433,050. Applicants respectfully request reconsideration.

Claims 17, 23, 76 and 80-85 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 30 and 34-55 of copending Application No. 11/142,866. Applicants respectfully request reconsideration.

The Examiner states that since both applications have one inventor in common a non-statutory double-patenting rejection is properly maintained. The Examiner has disagreed with Applicants' arguments that common ownership means wholly owned by the same person or organization. Applicants respectfully request reconsideration.

Applicants previously argued that the double patenting rejection was not appropriate because the twp applications are not "common owned". Common ownership is defined in MPEP 706.02(l). MPEP 706.02(l) states that the "term 'common ownership' means wholly owned by the same person(s) or organization(s) at the time the invention was made." The instant patent application is owned by four entities and US Patent Applications No. 10/832,257; 11/142,866 and 10/433,050 are owned by only one of the four entities. The Examiner has dismissed these arguments because the definition of "common ownership" is found in a section of the MPEP (706.02(I)) which "is directed to issues having to do with establishing common ownership under 35 USC 103(c) and does not directly address the particular problems of dual ownership of two or more patents to the same or obvious variations of the same invention, as is the case here." (Office Action page 7). However, MPEP 706.02(I) describes common ownership with respect to obviousness under 35 USC 103(c) as well as double patenting (See MPEP 706.02(I)(2) & (3)). Additionally, the case law supports Applicants' interpretation that double patenting requires common ownership. For instance the court in *In re Longi* (759 F.2d 887, 896, 225 USPQ 645, 651 (Fed. Cir. 1985)) addressed the issue that a double patenting rejection is appropriate over a patent having a different inventive entity if common ownership existed. Additionally Applicant is not aware of a definition of common ownership in the case law or the MPEP that contradicts the definition of common ownership found in MPEP 706.02(I).

As the Examiner is undoubtedly aware, the appropriate legal analysis for obviousness type double patenting requires a determination that the differences between the claimed inventions would

have been obvious to one of ordinary skill in the art. The court in *General Foods Corp. v. Studiengesellschaft Kohle MbH*, (972 F.2d 1272, 1279, 23 USPQ2d 1839, 1844 (Fed. Cir.. 1992)) has stated that "the determining factor in deciding whether or not, there is double patenting is the existence vel non of *patentable differences* between two sets of claims." "It is important to bear in mind that comparison can be made only with what invention is claimed in the earlier patent, paying careful attention to the rules of claim interpretation to determine what invention a claim defines and not looking to the claim for anything that happens to be mentioned in it as though it were a prior art reference." (*General Foods*, 972 F.2d at 1280.). In General Foods the court found that an issued claim that included several elements did not render obvious, under the judicially created doctrine of double patenting, a claim to a single element within the combination. Specifically the court stated that "[a]nything less than a process with all 9 steps is not what is claimed, and is, therefore, not patented. Claims must be read as a whole in analyzing a claim of double patenting" and "this concept violates the fundamental rule of claim construction, that what is claimed is what is *defined by the claim taken as a whole*, every claim limitation (here each step) being material." (*General Foods*, 972 F.2d at 1278-80.)

In the instant application the claimed invention is directed to methods for mediating RNA interference of an mRNA in a cell *in vitro* by introducing in to the cell double stranded RNAs of 21-23 nucleotides in length that mediate RNA interference of an mRNA wherein the dsRNA is in the form of two separate strands which are not covalently linked.

The claims of the instant application encompass the use of a genus of double stranded RNA having certain structural features, *e.g.*, length, complementarity with the target mRNA, two separate strands not covalently linked, coupled with the functional ability to mediate RNAi. The claims of US 10/832257 are also directed to methods of mediating RNA interference using double stranded RNAs, however, the claims of US 10/832257 feature methods of use of a different genera of RNAs as compared to the genus of double stranded RNAs useful according to Applicants' claimed methods. In particular, the claims of US 10/832257 feature methods of using double stranded RNAs wherein each strand is 19-23 or 19-25 nucleotides in length, at least one strand having a 3'overhang of 1-5 or 1-3 nucleotides. The claims of the instant invention are silent as to the

occurrence of a 3' overhang, whereas the claims of US 10/832257 feature methods of using double stranded RNAs which must include a 3' overhang of 1-3 or 1-5 nucleotides in length and do not include the limitation that the RNA is in the form of two separate strands which are not covalently linked.

The claims of US 10/433050 and 11/142866 are not directed to methods for mediating RNA interference. Rather, the claims of US 10/433050 are directed to isolated dsRNA and the claims of US 11/142866 are directed to methods of stabilizing dsRNA. A method of mediating RNAi would not have been obvious in view of isolated dsRNA and methods for stabilizing dsRNA. Both claim sets also require the use of 3' overhangs and do not include the limitation that the RNA is in the form of two separate strands which are not covalently linked.

The fact that there may be overlap between the claims does not establish that the pending claims are obvious variants of the claims of US 10/832257, 10/433050 and 11/142866. At the time of the instant application, March 2000, one of skill in the art would not have modified the methods claimed in US 10/832257, 10/433050 and 11/142866 to arrive at the methods claimed in the instant application. In particular, the skilled artisan would not have been motivated, based on the teachings of the US 10/832257, 10/433050 and 11/142866 claims directed to double stranded RNAs having '3 overhangs of 1-3 or 1-5 nucleotides, to arrive at the claimed invention featuring methods of using the claimed genus of double stranded RNAs in the form of two separate strands which are not covalently linked because at the time of the invention there was no teaching that such molecules having the recited structural features were a desirable genus of RNAi-mediating molecules. By relying on a portion of the claim rather than all of the elements the examiner is using the claim as though it were the prior art rather than determining what the claim discloses as a whole and determining if they are the same invention.

Accordingly, Applicants request withdrawal of the rejection in view of US Patent Applications No. 10/832,257; 11/142,866 and 10/433,050.

Claims 17, 20-23, 76 and 80-85 have been provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 76-78, 81, 86-88, 91, 108, 110, 112, 115-120 and 124-177 of copending Application No. 09/821,832.

Although, Applicants disagree with the rejection, terminal disclaimers signed by each of the co-owners are submitted herewith to overcome the rejection. It is believed that the rejection should be withdrawn.

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, the Director is hereby authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 23/2825, under Docket No. W0571.70010US03.

Dated:

Respectfully submitted,

By_____
Helen C. Lockhart
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EXHIBIT 4

DOCKET NO.: W0571.70010US03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tuschl et al.
Serial No.:	10/255,568
Confirmation No.:	2920
Filed:	September 26, 2002
For:	RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE
Examiner:	Amy Hudson Bowman
Art Unit:	1635

Certificate of Mailing Under 37 CFR 1.8(a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: January 5, 2007

Sharon R. Lloyd

Sharon R. Lloyd

AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INTRODUCTORY COMMENTS

In response to the Office Action dated September 6, 2006, rejecting claims 17-25 and 51-56, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1-16. (Canceled)

17. (Currently Amended) A method of mediating RNA interference of mRNA of a gene in a cell or organism comprising:

(a) introducing double stranded RNA of from about 21 to about 23 nucleotides in the form of two separate strands ~~which targets~~ that has sequence correspondence to the mRNA of the gene for degradation into the cell or organism and mediates RNA interference by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within the region of sequence correspondence with the RNA, and wherein the mRNA is mammalian cellular mRNA or viral mRNA;

(b) maintaining the cell or organism produced in (a) under conditions under which degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.

18. (Original) The method of Claim 17 wherein the RNA of (a) is a chemically synthesized RNA or an analog of naturally occurring RNA.

19. (Canceled).

20. (Currently Amended) A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising:

(a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby producing a combination;

(b) maintaining the combination produced in (a) under conditions under which the double- stranded RNA is processed to double stranded RNA of from about 21 to about 23 nucleotides, thereby producing double stranded RNA of from about 21 to about 23 nucleotides;

(b) isolating double stranded RNA of from about 21 to about 23 nucleotides produced in (b);

(c) introducing RNA isolated in (c) into the cell or organism; and

(d) maintaining the cell or organism produced in (d) under conditions under which degradation of mRNA of the gene occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.

21. (Original) The method of Claim 20, wherein the soluble extract is derived from syncytial blastoderm Drosophila embryos.

22. (Original) The method of Claim 20, wherein the RNA is isolated using gel electrophoresis.

23. (Currently Amended) A method of mediating RNA interference of mRNA of a gene in a cell or organism in which RNA interference occurs, comprising: (a) introducing into the cell or organism double stranded RNA of from about 21 to about 23 nucleotides in the form of two separate strands that mediates RNA interference of mRNA of the gene by directing cleavage of the mRNA to which it corresponds, wherein cleavage is directed within a region of sequence correspondence with the RNA, thereby producing a cell or organism that contains the RNA, and wherein the mRNA is mammalian cellular mRNA or viral mRNA and (b) maintaining the cell or organism that contains the RNA under conditions under which RNA interference occurs, thereby mediating RNA interference of mRNA of the gene in the cell or organism.

24. (Original) The method of claim 23, wherein the RNA of from about 21 to about 23 nucleotides is chemically synthesized RNA or an analog of RNA that mediates RNA interference.

25-75. (Canceled).

76. (New) The method of claim 17, wherein the mRNA is human mRNA.

77. (New) The method of claim 17, wherein the RNA is chemically synthesized RNA.

78. (New) The method of claim 17, wherein one or more nucleotides of the RNA are a non-naturally occurring nucleotide or deoxyribonucleotide or non-standard nucleotides.

79. (New) The method of claim 17, wherein the RNA is from about 21 to 24 nucleotides in length.

80. (New) The method of claim 17, wherein the RNA is from about 21 to 23 nucleotides in length.

REMARKS

Claims 1-75 were previously pending with claims 1-16, 26-50 and 57-75 withdrawn from consideration and claims 17-25 and 51-56 under examination. Claims 1-16, 19 and 25-75 have been canceled. Cancellation of these claims is made without prejudice to further prosecution. Applicants reserve the right to pursue claims to the same or similar subject matter in future applications. Applicants have amended claims 17, 20 and 23 and added new claims 76-80. Thus, claims 17-18, 20-24 and 76-80 are pending and under examination. No new matter has been added.

Claim Rejections – 35 USC § 112

Claims 17-25 and 51-56 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. According to the Examiner, the specification as filed does not enable the claimed methods of mediating RNA interference. The Examiner has cited several references to demonstrate the unpredictability of RNAi *in vivo*. It is concluded that "To practice the claimed invention, one of skill in the art would have to *de novo* determine the stability of the antisense molecule in vivo, delivery of the antisense molecule to the whole organism, specificity to the target tissue in vivo, dosage and toxicity *in vivo*, and entry of the molecule into the cell in vivo and the effective action therein." (Office Action page 5).

It would not have required undue experimentation for one of skill in the art to practice the methods of claims 17-18 and 20-25 in view of the teachings of the specification and what was known in the art at the time the patent application was filed. Claims 19 and 51-56 have been canceled and will not be addressed further. In order to advance prosecution, Applicants have amended each of the claims to clarify the mechanism of the claimed methods of mediating RNA interference and to recite that double stranded RNA is introduced into the cell.

a. Lack of prima facie rejection for non-enablement

A prima facie case for lack of enablement of the claims has not been presented. Under 35 U.S.C. §112, first paragraph, the Examiner has the "initial burden of setting forth a reasonable

explanation as to why the scope of protection provided by [the claims] is not adequately enabled by the description of the invention provided in the specification." In re Wright, 999 F.2d 1557 (Fed. Cir. 1993). Specifically, in In re Brana, 51 F.3d 1560, 1566 (Fed. Cir. 1995), it was held that: 'Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility.' Additionally, the court stated that in the absence of a reason to doubt the objective truth of the teachings contained in the specification, the methods of making and using the claimed invention must be taken as complying with the requirements of §112, first paragraph.

Applicants have asserted that the claimed methods are enabled and presented sufficient description in the specification to enable one of skill in the art at the time the application was filed to practice the method. Applicants have not presented unsupported ideas without providing guidance in the specification. Rather, Applicants have made an important discovery, based on significant amounts of data, regarding a class of molecules that have enormous utility for a range of different applications in vitro and in vivo. The data is included in the patent application. The invention is described throughout the specification. Applicants have provided guidance on the structure of the molecule and how to use it.

As mentioned above, the Examiner concluded that "To practice the claimed invention, one of skill in the art would have to *de novo* determine; the stability of the antisense molecule in vivo, delivery of the antisense molecule to the whole organism, specificity to the target tissue in vivo, dosage and toxicity *in vivo*, and entry of the molecule into the cell in vivo and the effective action therein." (Office Action page 5). Applicants submit that the Examiner has not shown any of these factors to be sufficient to establish a prima facie case of lack of enablement and that the invention would require undue experimentation.

"the stability of the antisense molecule in vivo": The Examiner has provided no evidence that stability issues would prevent mediation of RNAi in a cell or organism. While highly stable constructs may be desirable for various reasons, the PTO has advanced no evidence to suggest that the method as claimed could not be employed to do what is recited, *i.e.*, mediate RNAi in a

cell or organism to a useful degree. Whether additional research is required to optimize the method or make it commercially viable for widespread adoption is irrelevant to the issue of whether the method as claimed can be made to work as claimed without undue experimentation. Furthermore, a significant amount of research prior to the filing of the instant application was focused on stabilization of nucleic acids that might be used as potential therapeutic agents. Thus, if stabilization was desired, it could easily be achieved by art-recognized means.

"delivery of the antisense molecule to the whole organism" and "specificity to the target tissue in vivo": Generally a compound is delivered using conventional modes to a target tissue. For instance if an RNAi therapeutic agent were to be delivered to a tumor it might be delivered systemically or directly to the tumor site. Those of skill in the art are well aware of methods for delivering compounds based on a particular target location or disease. Moreover, the Patent Office has supplied no evidence that one skilled in the art could not deliver the recited constructs as claimed without undue experimentation.

"dosage and toxicity *in vivo*": MPEP 2164.01(c) states "For example, it is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation. If one skilled in the art, based on knowledge of compounds having similar physiological or biological activity, would be able to discern an appropriate dosage or method of use without undue experimentation, this would be sufficient to satisfy 35 USC 112, first paragraph. The applicant need not demonstrate that the invention is completely safe." No indication is provided by the Examiner why the selection of a dosage would require undue experimentation. Applicants are not aware of specific teachings of serious problems with identifying appropriate dosages or toxicity that interfere with the use of RNAi technology or which have caused undue experimentation. The PTO has not provided evidence of specific teachings of serious problems with identifying appropriate dosages or toxicity that interfere with the use of RNAi technology or that undue experimentation was required. Moreover, in order for a claimed invention to be enabled, the standard is not whether or not experimentation is necessary to practice the claimed invention. Rather, the standard is whether or not the experimentation necessary to practice the claimed invention is undue (See In re Wands, 858 F.2d, 731 at 737 (Fed. Cir. 1988) and MPEP 2164.02). Thus, enablement is not

precluded by the necessity for some experimentation, and a considerable amount of experimentation is permitted. In re Wands, supra. the Examiner appears to suggest that enablement is lacking unless one of skill in the art could predict, *a priori*, whether introduction of RNA *in vivo* would result in successful RNA interference (Office Action page 5). Such a standard would seem to preclude any experimentation. If the Examiner is aware of any legal basis for a standard that requires complete predictability, she is invited to provide it.

"entry of the molecule into the cell in vivo and the effective action therein": It is unclear what is meant by this statement. If the Examiner is aware of some teaching that would demonstrate problems with RNA molecules having an effective action within cells, she is requested to provide that teaching if this basis of rejection is to be maintained.

It is also noted that the claims have been amended to recite use of double-stranded RNA, thereby obviating any basis for rejecting the claims on grounds related to alleged unpredictability associated with use of antisense technology.

b. *Cited References*

The Examiner has cited several post filing references (Scherer et al., Mahato et al., and Zhang et al.) to support the conclusion that the claimed method was unpredictable at the time the application was filed.

The Examiner has pointed to the teachings in Scherer that indicate that certain challenges, in particular effective application, delivery, stability minimization of off-target effect and identification of sensitive sites in the target RNAs must be met before any antisense molecule or RNAi molecule "can become widely accepted as a therapeutic tool." (Office Action page 4).

The teachings of Scherer are not sufficient to support the unpredictability of the claimed invention. Scherer et al is a review article describing sequence specific knockdown of mRNA. The reference describes different techniques for knock down and presents advances and challenges proposed or encountered. Any potential therapeutic compound faces challenges prior to becoming widely accepted as a therapeutic tool. The fact that a compound faces challenges

before becoming widely accepted as a therapeutic tool is not a sufficient basis for demonstrating that the claimed invention lacks enablement. Patentability does not require that the claimed invention be perfected to the degree necessary to become a widely accepted therapeutic tool.

Additionally, Scherer et al provides teachings that are consistent with the enablement of the claimed invention. For instance Scherer et al teaches:

> "RNAi has emerged as a potent mechanism to specifically knockdown mRNA transcripts to a few percent of their original levels by most methods of detection. RNAi appears to be more potent than antisense RNAs, ribozymes or RNAzymes for targeted message destruction, presumably because it exploits cellular machinery that efficiently directs the antisense component to the target mRNA for site-directed cleavage." (page 1461, 1st column 2nd full paragraph.)

> "Alternatively, siRNAs provide an additional antisense-based tool that may be even more powerful combined with the other nucleic acid-based therapies. The discovery of RNAi has certainly accelerated the pace at which targeted post-transcriptional gene silencing is being applied as a tool for identifying gene function and as a therapeutic agent." (page 1463, 2nd column 2nd full paragraph.)

Mahato et al, like Scherer et al, is a review article describing the advances and challenges in sequence specific nucleic acid inhibitors. As pointed out by the Examiner, Mahato et al describe successful work in the field. For instance on page 16 section 4.3 the authors summarize several research studies that show promise, including one study that "suggest that siRNAs can be applied for tumour-specific gene therapy to reverse the oncogenic phenotype" and another which suggests "the therapeutic promise of siRNA to prevent liver injury by protecting hepatocytes from cytotoxicity." They also point out some challenges that are faced in developing optimized therapeutic methods using this technology. The fact that a compound faces challenges during development as a therapeutic is not a sufficient basis for demonstrating that the claimed invention lacks enablement. Patentability does not require that the claimed invention be perfected to the degree necessary to become a widely accepted therapeutic tool.

Zhang et al is a 2004 review article describing a compilation of research studies on siRNA. Zhang et al concludes in the last paragraph of the paper by stating that challenges in the development of siRNAs still exist but that siRNA "holds great promise as a tool for validating

drug targets and treating disease." The teachings of Zhang et al are not inconsistent with the enablement of claimed invention.

Each of the cited references is a post-filing reference. It is taught in the MPEP (section 2164.05(a)) that "In general, the examiner should not use post-filing date references to demonstrate that the patent is non-enabling. Exceptions to this rule could occur if a later-dated reference provides evidence of what one skilled in the art would have known on or before the effective filing date of the patent application." In the instant case, the examiner has not identified any teachings that are evidence of what one skilled in the art would have known on the filing date.

The teachings of the cited references are directed to RNAi technology, as well as antisense, but they do not establish unpredictability of the claimed invention at the time the patent application was filed. Aside from the quotes by the Examiner, portions of these teachings actually illustrate examples of successful applications of RNAi technology. It is correct that these reviews also identify the area where improvements in the technology may be desirable. Like any other nascent technology, RNAi too, presents many opportunities for improvements and development, and although many improvements and alternative delivery strategies have been developed since the invention was made by the Applicant, the invention can be practiced as claimed and described in the instant application. The CAFC has explicitly stated, "Enablement does not require the inventor to foresee every means of implementing an invention at pains of losing his patent franchise. Were it otherwise, claimed inventions would not include improved modes of practicing those inventions. Such narrow patent rights would rapidly become worthless as new modes of practicing the invention developed, and the inventor would lose the benefit of the patent bargain." *Invitrogen Corp. v. Clontech Laboratories, Inc.*, 429 F.3d 1052 at 1071 (Fed. Cir. 2005). Patentability is not precluded merely because optimization or improvements for widespread therapeutic use may be desired.

Claim Rejections – 35 USC § 102

The Examiner has also rejected claims 17-19 under 35 USC 102 as being anticipated by

Baracchini et al (US 5,801,154). The claims have been amended to clarify that the RNA being administered to the subject is double stranded. It is believed that the amendment to the claim language should be sufficient to distinguish the claimed invention from Baracchini et al. Baracchini et al describes single stranded antisense oligonucleotides. Thus, claims 17-18 (claim 19 is canceled) are not anticipated by Baracchini et al.

Claims 17-20 and 23-25 have been rejected under 35 U.S.C. 102(b) as being anticipated by Agrawal et al. (WO 94/01550) as further evidenced by Bridge et al.

Amended claims 17-18, 20-24 and new claims 76-80 are not anticipated by Agrawal et al because each include a limitation not found explicitly or inherently in Agrawal et al. The Examiner has stated that Agrawal et al describe polymers that may be ribonucleotides and which have partial or complete double stranded regions. The Examiner further states that in view of the teachings of Bridge et al (Nature Genetics, 2004, v. 34, p.263) it is clear that the molecules of Agrawal would inherently be processed into siRNAs of 21 nucleotides in length. The Examiner then concludes that the molecules of Agrawal inherently anticipate the claimed invention when introduced into cells. Claims 17, 20 and 23 have been amended to clarify that the RNA being introduced into the cell or organism is double stranded and is in the form of two separate strands. It is believed that the amendment to the claim language should be sufficient to distinguish the claimed invention from Agrawal et al. Agrawal et al does not disclose the introduction into a cell of a double stranded RNA of about 21-23 nucleotides in length. Even if the material of Agrawal et al were processed to produce a double stranded siRNA within the cell, which we do not concede, that point is not relevant to anticipation of the claim which recites a step of introduction into the cell. Thus, Applicants respectfully submit that the rejection of claims 17-20 and 23-25 should be withdrawn.

Claim Rejections – 35 USC § 103

Claims 17-25 and 51-56 have been rejected as being obvious under 35 USC 103 over Agrawal et al in view of Fire et al. The rejection is based on the same arguments that were presented for the rejection under 35 USC 102. For each of the reasons cited above with respect

to the teachings of Agrawal, the rejection under 35 USC 103 should also be withdrawn. The Examiner has cited Fire et al to provide missing teachings relating to methods for introducing the RNA using recombinant DNA (claims 51-56). Claims 51-56 have been canceled without prejudice to further prosecution. Thus, Applicants respectfully submit that the rejection should be withdrawn.

Double Patenting

Claims 17-20 and 23-25 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 50-52, 54, 55, 68-72, 74, 87-91, 93, 94, 107-111, 113, 126, and 127 of copending Application No. 10/832,257.

As the Examiner is aware, if provisional obviousness-type double patenting rejections in two applications are the only rejections remaining in those applications, the Examiner should withdraw the rejection in the earlier filed application, *i.e.*, the instant case, thereby permitting that application to issue as a patent (MPEP 804(I)(b)(1)).

CONCLUSION

In view of the above amendment, Applicants respectfully submit that the pending application is in condition for allowance.

Dated: January 5, 2007

Respectfully submitted,

By _____

Helen Lockhart
Registration No.: 39,248
WOLF, GREENFIELD & SACKS, P.C.
Federal Reserve Plaza
600 Atlantic Avenue
Boston, Massachusetts 02210-2206

EXHIBIT 5

From: Robert Millman [rmillman@alnylam.com]
Sent: Thursday, December 07, 2006 2:52 PM
To: Lockhart, Helen
Subject: RE: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03
Thank you this is better.

From: Lockhart, Helen [mailto:Helen.Lockhart@WolfGreenfield.com]
Sent: Thursday, December 07, 2006 10:35 AM
To: Robert Millman
Cc: Robert Murray; mkitts@rothwellfigg.com; Ann Saitta; Monica Gerber
Subject: RE: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03

Robert

Thank you for your comments.

Based on the publicly available information in PAIR regarding 10/832,257 it appears that Tom Tuschl is an inventor of US 10/832,257. Also the earliest priority date that I could find for 10/832257 is 3/30/01, one year after the earliest priority date for 10/255,568. Please let me know if this is incorrect. If Tuschl is an inventor, I cannot argue that the applications are not commonly invented. According to the MPEP double patenting may exist between two applications that have a single inventor in common. Identical inventive entities and ownership is not required.

My intent in presenting the argument was to avoid making any substantive arguments that might characterize the scope of what is taught in 10/832257. I believe that my proposed response is in fact correct and provides no misleading arguments to the PTO. However it is not essential for me to make all of these arguments in view of this provisional rejection. In view of your objections, I propose to replace my prior arguments with the arguments presented below. Please let me know if you have any objections to this proposed response.

regards
Helen

Proposed Revised Response:

Double Patenting

Claims 17-20 and 23-25 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 50-52, 54, 55, 68-72, 74, 87-91, 93, 94, 107-111, 113, 126, and 127 of copending Application No. 10/832,257. As the Examiner is aware, if provisional obviousness-type double patenting rejections in two applications are the only rejections remaining in those applications, the examiner should withdraw the rejection in the earlier filed application, *i.e.*, the instant case, thereby permitting that application to issue as a patent.

From: Robert Millman [mailto:rmillman@alnylam.com]
Sent: Monday, December 04, 2006 3:23 PM
To: Lockhart, Helen
Cc: Robert Murray; mkitts@rothwellfigg.com; Ann Saitta
Subject: RE: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03
Importance: High

Helen:

We do not approve this Response and do not agree that it should be filed as proposed.

Specifically, 10/832,257 cannot be cited as a same invention double patenting or an obvious-type double patenting for this case since 1) it is available as prior art, 2) it is not commonly invented, 3) it is not commonly owned.

The only way you should respond to this rejection is to point out that the application in question is not commonly invented nor owned.

Your proposed response is incorrect and provides misleading information to the USPTO which would be detrimental to any patent that ultimately issues from this case.

Robert Millman
Chief IP Counsel
Alnylam Pharmaceuticals

From: Ann Saitta
Sent: Monday, December 04, 2006 3:17 PM
To: Robert Millman
Subject: FW: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03

From: Lockhart, Helen [mailto:Helen.Lockhart@WolfGreenfield.com]
Sent: Wednesday, November 29, 2006 12:22 PM
To: Amina Hamzaoui
Cc: Patricia.Tuft@umassmed.edu; Decker, Lisa; Shawna Vogel; Andy Roth DE-HILDEN; dbartel@wi.mit.edu; Ana Ward; bsauerbrei@sial.com; email@weickmann.de; ttuschl@mail.rockefeller.edu; phillip.zamore@umassmed.edu; sharppa@mit.edu; djm@lahive.com; rmurray@rothwellfigg.com; bjarrell@choate.com; Ann Saitta; Monica Gerber
Subject: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03

U.S. Patent Application No. 10/255,568 Entitled: RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE Filing Date: September 26, 2002 Inventor(s): Tuschl et al.
Your Ref. No.: WHI00-06CON: MIT 8768W; UMMC01-36; GI2716 ZTM; W0571.70010US03

Dear Amina

Attached is a draft response to Office Action for the above-identified patent application. The first deadline for response is December 6, 2006. It is possible to take up to three months of extension if necessary. Please review the draft and let me know if you have any questions or comments or if you need additional time to review. If I do not hear that additional time is required for review of the draft I will file it on December 6, 2006 with any necessary changes incorporated.

Please let me know if you have any questions or comments.

regards
Helen

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<http://www.wolfgreenfield.com/>
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